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**ANALYTICAL METHOD FOR
CONCENTRATION OF TRACE ORGANICS
FROM WATER**

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APRIL 1981

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) An efficient and rapid method is described for the concentration of trace organics from water. The compounds tested were chosen from the primary and secondary products of the microbial transformation of 2,4,6-trinitrotoluene (TNT). The four compounds covered a range of polarities (2,4-diamino-6-nitrotoluene, TNT, 2-amino-4,6-dinitrotoluene and 2,2',6,6'-tetranitro-4,4'-azoxytoluene) and were tested at concentrations down to 1 ppb. Solutions were extracted with SEP-PAK C-18 cartridges (Waters Assoc.) and quantitized by high performance liquid		

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20. Abstract Cont'd

chromatography. Efficient recoveries were obtained for three compounds while the most polar compound, 2,4-diamino-6-nitrotoluene, showed low recovery. Amounts down to 30 ng were routinely detected.

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PREFACE

Current guidelines require the detection of organic compounds in wastewater down to ppb levels. Included under these requirements are Army specific wastes like 2,4,6-trinitrotoluene (TNT) which contaminates wastewaters at manufacturing and loading facilities. During studies on microbiological transformations of TNT-surfactant complexes in soil and water (Project No. 03210502040), it was necessary to develop a method of concentration and detection of trace quantities of TNT and its biotransformation products in water. This report describes procedures to meet this requirement. The TNT reduction products used in this study were supplied by Dr. John C. Hoffsomer, US Naval Surface Weapons Center, Silver Spring, MD.

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ANALYTICAL METHOD FOR CONCENTRATION OF TRACE ORGANICS FROM WATER

INTRODUCTION

Current guidelines for acceptable levels of discharge of many organic compounds into waste waters require detection of concentrations to the ppb level. Army specific munition wastes like 2,4,6-trinitrotoluene (TNT) are included under these requirements. At these low levels the organic compounds must be concentrated for reliable quantitation by standard analytical procedures. Concentration may be accomplished by a number of methods including evaporation, lyophilization, solvent extraction (separatory funnel or 24-hour continuous) and column absorption.^{1,2,3} Solvent extraction is the method of choice for many of these compounds, but large solvent volume handling problems, poor extraction efficiencies, lengthy extraction periods, the hazardous nature of some solvents and high costs are serious drawbacks.

During studies on the biodegradation of TNT-surfactant complexes, it was necessary to determine low concentrations of a number of TNT reduction products that could arise during microbiological transformations of TNT.⁴ TNT and three of these reduction products were used in this study.

¹American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1975. Standard Methods for the Examination of Water and Wastewater. 4th Ed. Washington, DC.

²Environmental Protection Agency. 1973. Handbook for Monitoring Industrial Wastewater. Technology Transfer. Washington, DC.

³Environmental Protection Agency, Environmental Monitoring and Support Laboratory. 1977. Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants. Cincinnati, OH.

⁴N. G. McCormick, F. E. Feeherry and H. S. Levinson. 1976. Microbial Transformation of 2,4,6-Trinitrotoluene, and other Nitroaromatic Compounds. Appl. Environ. Microbiol. 31: 949-958.

Objective

The objective of this work was to develop a method for rapid and efficient concentration and determination of trace organics in water down to the 1 ppb level.

MATERIALS AND METHODS

High Performance Liquid Chromatography (HPLC)

Quantitative analysis was performed on a Waters Liquid Chromatograph equipped with Model 6000A solvent pumps, Model 450 variable wavelength detector at 230 nm, Data Module and Model 720 System Controller. The mobile phase was methanol/water at ratios indicated on Tables. Injections were 50 μ L onto a 30 cm x 3.9 mm ID Waters μ Bondapak reverse phase C-18 column at room temperature. The flow rate was 2.5 mL per min.

Sample Preparation

Compounds to be analyzed were concentrated by passing dilute solutions through SEP-PAK cartridges, a commercial product of Waters Associates, Milford, MA. The C-18 SEP-PAK with μ Bondapak packing, an octadecylsilane treated silica bonded phase, was used in all experiments. After washing the cartridge with methanol and water, 300-mL solutions were drawn through it at about 10 mL per min by suction. Compounds collected on the cartridge were then eluted with 5 mL methanol. The methanol was concentrated down to 0.5 mL with air at room temperature and analyzed directly by HPLC.

Compounds

Commercially available TNT was recrystallized. Dr. John C. Hoffsomer, US Naval Surface Weapons Center, Silver Spring, MD, supplied samples of 2,4-diamino-6-nitrotoluene (2,4 DA), 2-amino-4,6-dinitrotoluene (2A) and 2,2',6,6'-tetranitro-4,4'-azoxytoluene (4,4'Az).

RESULTS

Figure 1 illustrates an HPLC tracing for all four compounds using a methanol gradient from 40 to 78% in water. The compounds examined fall in a wide range of polarities so the efficiency of concentration could be examined for compounds of very different affinities. These chromatographic results indicate a relative order of polarity of 2,4DA>TNT>2A>4,4'Az.

In Table 1 results are presented for solutions of TNT from 1 to 100 ppb concentrated 600-fold by passage through SEP-PAK cartridges. These results are compared to a theoretical maximum determined from standard curves. About 70% recovery at 5 ppb and 80% at 10 and 100 ppb was found using this system. At 1 ppb, recovery was only about 29% but was reproducible. Without concentration, the limits of detection for TNT are about 25 ppb. Levels as low as 500 ppt were detected with SEP-PAKS, but results were only preliminary.

Table 2 presents the results for 2A. As with TNT, recoveries based on expected values derived from standard curves were about 80% for 10 and 100 ppb. At 1 ppb about 70% was recovered.

Low recoveries were obtained with 2,4DA, the most polar compound tested, (Table 3). Recoveries were no higher than about 11%.

The most nonpolar compound tested was 4,4'Az which was efficiently recovered at 3.2, 32.2 and 322.0 ppb levels. Between 73 and about 95% recoveries were obtained (Table 4).

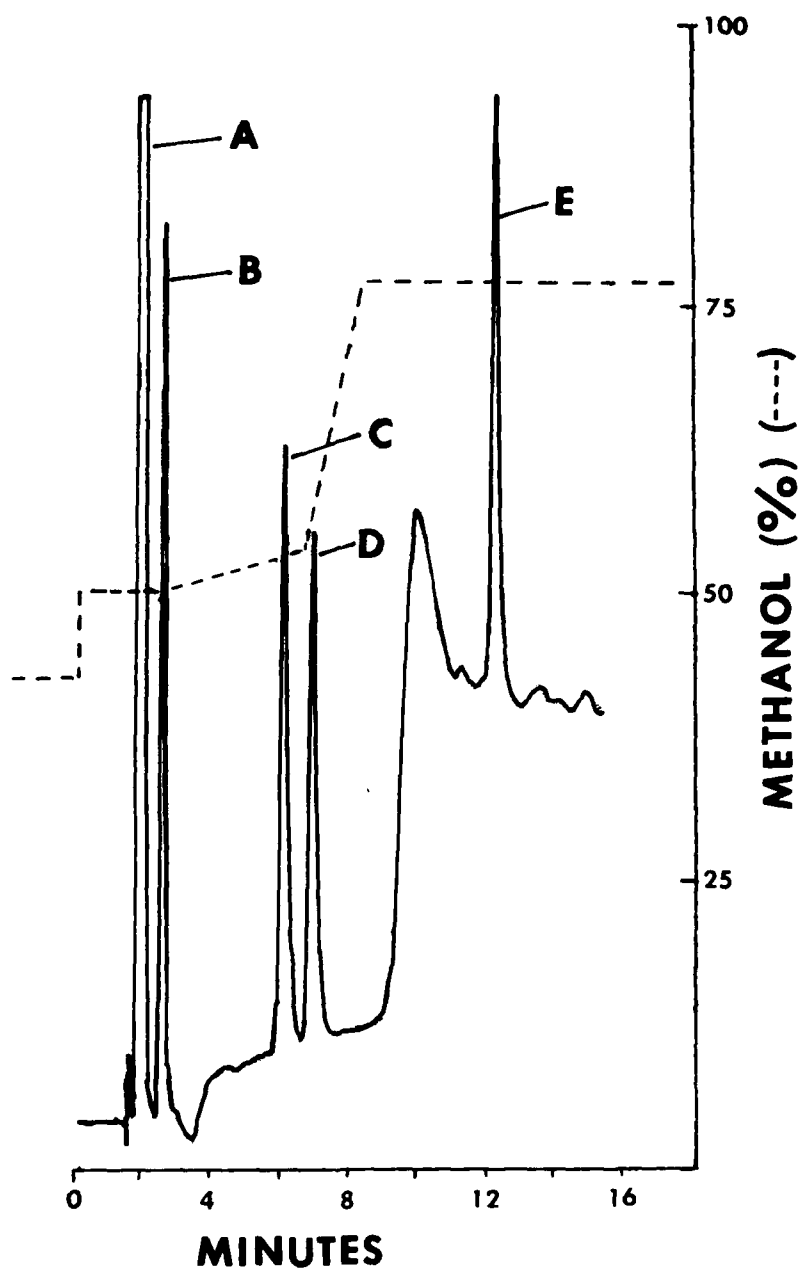


Figure 1. HPLC tracing with methanol gradient. Acetone (A), 2,4-diamino-6-nitrotoluene (B), 2,4,6-trinitrotoluene (C), 2-amino-4,6-dinitrotoluene (D) and 2,2', 6,6'-tetranitro-4,4'-azoxytoluene (E). 0.1 AUFS.

TABLE 1 Efficiency of Extraction of 2,4,6-trinitrotoluene

Solution Concentration (ppb)	Concentration Factor	Amount Injected ^a (μg)	\bar{x} (area) ± I.S.D. N = 5	Expected Values from Standard Curve ^b		Percent Recovery
				Conc. (ppm)	Area	
1	600	0.03	70.5 ± 10.3	0.6	240.9	29.3
5	600	0.15	591.2 ± 65.3	3.0	1000.0	69.1
10	600	0.30	1751.6 ± 94.0	6.0	2224.8	78.7
100	600	3.00	19129.0 ± 1226.1	60.0	24269.7	78.8

a. 50 μl Injections.

b. 50% Methanol/Water.

TABLE 2 Efficiency of Extraction of 2-amino-4,6-dinitrotoluene

Solution Concentration (ppb)	Concentration Factor	Amount Injected ^a (μg)	\bar{x} (area) ± I.S.D. N = 3	Expected Values from Standard Curve ^b		Percent Recovery
				Conc. (ppm)	Area	
1	600	0.03	339.0 ± 44.8	0.6	487.7	69.5
10	600	0.30	3220.0 ± 163.0	6.0	4123.0	78.1
100	600	3.00	32851.7 ± 720.3	60.0	40375.9	81.4

a. 50 μl Injections

b. 50% Methanol/Water

TABLE 3. Efficiency of Extraction of 2,4-diamino-6-nitrotoluene

Solvent Concentration (ppb)	Concentration Factor	Amount Injected ^a (μg)	\bar{x} (area) \pm IS.D. N = 3	Expected Values from Standard Curve ^b		Percent Recovery
				Conc. (ppm)	Area	
1	600	0.03	20.0 \pm 5.3	0.06	387.7	5.2
10	600	0.30	204.0 \pm 44.9	6.0	3681.4	5.5
100	600	3.00	4014.7 \pm 678.1	60.0	36658.4	10.9

a. 50 μl Injections

b. 40% Methanol/Water

TABLE 4. Efficiency of Extraction of 2,2',6,6'-tetranitro-4,4'-azoxytoluene

Solution Concentration (ppb)	Concentration Factor	Amount Injected ^a (μg)	\bar{x} (area) \pm IS.D. N = 3	Expected Values from Standard Curve ^b		Percent Recovery
				Conc. (ppm)	Area	
3.2	600	0.096	444.0 \pm 27.2	1.9	608.4	73.0
32.2	600	0.960	5992.3 \pm 171.5	19.3	6324.5	94.7
322.0	600	9.600	52806.3 \pm 2907.2	193.2	63420.1	83.3

a. 50 μl Injections

b. 75% Methanol/Water

DISCUSSION

SEP-PAK cartridges can be successfully used for the concentration and subsequent determination of compounds present in aqueous systems in trace amounts. The cartridges have valuable application in the field of explosives analysis and probably for a variety of other types of organic compounds.

The main advantages of this system are the rapidness of sample preparation, reduced handling of samples, reduction in the quantities of solvents, lower cost, sensitivity and reproducibility. Also the disposable nature of the cartridges after a single use is practical. The major drawback is concentration of interfering compounds if present, although the eluent from the cartridge can be extracted with a small volume of appropriate solvent for purification.

The results show that as polarity increases, the efficiency of extraction decreases. Satisfactory recoveries were obtained with TNT, 2A and 4,4'Az, but recoveries were poor with 2,4DA. Possibly a more efficient extraction of 2,4DA could be obtained by using silica SEP-PAKS (Waters Assoc.). With 300 mL aliquots about 30 ng of material was detectable after concentration and amounts as low as 15 ng at these volumes were also detected.

CONCLUSION

We have demonstrated that SEP-PAK C-18 cartridges can be successfully utilized for the concentration and determination of trace organics from aqueous systems. In general, the more nonpolar the compound, the higher the recovery. The method is rapid, reproducible, efficient, and requires less solvent and sample handling than standard methods. It is particularly adaptive to the quantitation of explosives and their biodegradation products.

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